PROBING SINGLE MOLECULES OF DNA AT MULTIPLE LENGTH SCALES

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Current DNA analysis techniques are often focused on a single length scale. For example, fluorescent hybridization probes are used to physically map sequence-tagged sites, and *in vitro* synthesis, to sequence individual base pairs. While all of these techniques have proved satisfactory, there is an overwhelming desire to probe DNA directly at multiple length scales without such advanced preparations such as polymerase chain reaction amplification. If this could be accomplished, then DNA analysis would no longer be confined to the walls of the laboratory. Our goal is to probe DNA at multiple length scales—from a few hundred kilobases to single-base pairs—in a *single-assay format* using a combination of both optical and electronic detection methods. Specifically, we are using fluorescent hybridization probes to locate sequence-tagged sites on a single DNA molecule and scanning tunneling microscopy (STM) to sequence individual base pairs on that very same molecule.

Using molecular combing [1, 2], directed fluid-flow in microchannels [3], and microcontact printing [4], we are aligning and stretching single molecules of DNA into patterned arrays on gold-coated silicon substrates for subsequent imaging and analysis. Very briefly, we apply microcontact printing techniques to fabricate 1 ? m lines of self-assembled monolayers (SAMs) of alkanethiol onto gold-coated silicon substrates. We then place poly(dimethyl siloxane) (or PDMS)-fabricated microcapillaries on top of the patterned substrates top (the axis of the channels orthogonal to the lines of SAMs) and fill the capillaries with solutions containing DNA. After allowing sufficient time for the DNA molecules to spontaneously attach at one end to the hydrophobic lines of the patterned SAMs, we draw fluid out of the capillaries with a mild vacuum to thus stretch or “comb” the DNA. In this way, our defined approach allows for 1) the use of small quantities of DNA; 2) the parallel alignment of multiple species of DNA with precise control of spatial location; and 3) the use of multiple hybridization probes on adjacent lanes of DNA.

Currently, we are performing low-temperature STM measurements on patterned, stretched ? -DNA. Our goal is to differentiate individual DNA bases based on their spectroscopic electronic fingerprint. Already, we have been able to study the unique electronic states of other individual molecules with our STM technique [5]. It is therefore highly likely we will be able to probe DNA on the single molecule level with the STM. We will present preliminary results in this regard. If successful in our goals, we should be able to transfer our technique onto a single microchip and thus have a compact, rugged integrated device that is capable of direct, single-molecule probing.

References

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